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The effect of omega-3 fatty acid supplementation on plasma orexin A, plasma fatty acids, and anthropometric measurements in patients with narcolepsy

Makbule GEZMEN KARADAĞ[1], Meral AKSOY[2]

Aim: To determine the effect of omega-3 (n-3) fatty acid supplementation on plasma orexin A, plasma fatty acids, and anthropometric measurements in patients with narcolepsy.

Materials and methods: The study was performed on 17 males with narcolepsy and 14 healthy males between the ages of 21-55. An n-3 fatty acid supplement of 1.4 g/day was administered to the groups for 2 months. The anthropometric measurements of the individuals were taken at the onset and the end of trial, and orexin, fatty acids, and other biochemical parameters in the plasma were analyzed.

Results: While there was no statistically significant change in anthropometric measurements or the other body composition values of the patient group ($P > 0.05$), there was a statistically significant increase in the body weight, body mass index, waist-to-hip ratio, and body fat mass values (kg, %) of the control group ($P < 0.05$). There was no difference in the levels of plasma orexin A of the patients within or between groups in the pre- and poststudy measurements ($P > 0.05$). The poststudy plasma serotonin levels of the patients were lower and plasma insulin levels were higher than in the initial study ($P < 0.05$). There was a correlation between the plasma orexin A levels and plasma n-3 fatty acids in individuals in the control group at the onset of the study ($P < 0.05$). Depending on the increase of the fatty acids after the n-3 supplementation, a correlation between plasma orexin A levels and some fatty acids was observed ($P < 0.05$).

Conclusion: The data demonstrated that supplementation of n-3 fatty acid did not enhance the plasma orexin A level but did affect some other biochemical parameters.

Key words: Narcolepsy, sleep, orexin, n-3 fatty acid, body mass index, anthropometric measurements

Narkolepsili hastalarda omega-3 yağ asit desteğinin plazma oreksin A, plazma yağ asitleri ve antropometrik ölçümlere etkisi

Amaç: Bu çalışmada narkolepsili hastalarda omega-3 (n-3) yağ asit desteğinin plazma oreksin A, plazma yağ asitleri ve antropometrik ölçümlere etkisini saptamak amaçlanmıştır.

Yöntem ve gereç: Çalışma, 21-55 yaş arası 31 erkek birey (17 narkolepsili ve 14 sağlıklı) üzerinde yapılmıştır. Gruplara iki ay süresince günlük 1,4 g n-3 yağ asidi desteği verilmiştir. Çalışma başlangıcında ve sonunda bireylerin antropometrik ölçümleri alınmış plazmada oreksin, yağ asitleri ve diğer biyokimyasal parametreler analiz edilmiştir.

Bulgular: Çalışma sonunda, hasta grubun antropometrik ölçüm ve diğer vücut bileşim değerlerinde istatistiksel olarak önemli değişiklik yokken ($P > 0,05$), kontrol grubunun vücut ağırlığı, beden kitle indeksi, bel/kalça oranı, vücut yağ (kg, %) değerlerindeki artış istatistiksel açıdan önemli bulunmuştur ($P < 0,05$). Hastaların çalışma başı ve sonunda plazma oreksin A düzeylerinde hem grup içi hem de gruplar arasında fark gözlenmemiştir ($P > 0,05$). Yine hastaların plazma

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[1] Department of Nutrition and Dietetics, Faculty of Health Sciences, Gazi University, Ankara - TURKEY
[2] Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe University, Ankara - TURKEY
Correspondence: *Makbule GEZMEN KARADAĞ, Department of Nutrition and Dietetics, Faculty of Health Sciences, Gazi University, Ankara - TURKEY*
E-mail: mgezmen@gazi.edu.tr

seratonin düzeyleri çalışma başına göre, sonunda azalmış, insülin düzeyleri ise yükselmiştir (P < 0,05). Bireylerin grup içinde plazma n-6/n-3 oranında çalışma sonunda azalma olmasına karşın istatistiksel açıdan önemli değildir (P > 0,05). Plazma n-6/n-3 oranında gruplar arasında ise istatistiksel farklılık görülmüştür (P < 0,05). Kontrol grubundaki bireylerin çalışma başında plazma oreksin A düzeyi ile plazma n-3 yağ asitleri arasında korelasyon görülmüştür (P < 0,05). Yine çalışmada n-3 suplemantasyonu ardından yağ asitlerinin artmasına bağlı olarak plazma oreksin A düzeyi ile bazı yağ asitleri arasında korelasyon gözlenmiştir (P < 0,05).

Sonuç: Bu veriler; n-3 yağ asidi desteğinin plazma oreksin A düzeyini yükseltmediğini ancak diğer bazı biyokimyasal parametreleri etkilediğini göstermektedir.

Anahtar sözcükler: Narkolepsi, uyku, oreksin, n-3 yağ asitleri, beden kütle indeksi, antropometrik ölçümler

Introduction

Narcolepsy, known as a chronic sleep disorder, was defined by Westphal in 1877 and Gelineau in 1880. According to the USA Sleep Disorders Association, the diagnosis in the patient is established by excessive daytime sleepiness, cataplexy characterized by a sudden and bilateral loss of muscle tone with preserved consciousness, sleep paralyses, hallucinations, polysomnographic tests, and HLA DQB1*0602 genetic scanning. Due to the lower levels of orexin in patients with narcolepsy, diagnosis in these patients is also now established by examining their orexin levels (1,2). The yearly incidence of narcolepsy seen with cataplexy is 0.74 in 100,000, and the yearly incidence of narcolepsy without cataplexy is 1.37 in 100,000 (3). The pathophysiology of the disease is not completely known yet, but in the trials performed, it has been stated that it may derive from the imbalance existing between cholinergic and monoaminergic neurotransmitter systems (4,5). Although there is no direct treatment for the patients, it is possible to decrease the frequency of the symptoms (6,7). Drug administration (amphetamine-like stimulants, modafinil, tricyclic antidepressants, etc.) and psychiatric practices are often used in the treatment of narcolepsy (8).

Recently, orexin, a hypothalamic neuropeptide, was discovered; its possible roles in the pathology of narcolepsy have been indicated by various trials and it has been detected to play a role in sleep/wakefulness (9,10). It was stated that orexin neurons are very low in 85%-95% of patients with narcolepsy compared to nonnarcoleptic individuals (10,11).

Some hypothalamic neuropeptides, such as orexin, are generally affected by nutritional status and dietary food consumption (12-14). The amount of dietary fat intake and the types of fatty acids consumed could also change the neuropeptide expression (15,16). In a study by Wortley et al. (17), it was demonstrated that intralipid injections into the perifornical hypothalamus enhanced the levels of triglycerides and increased the orexin gene expression in rats. In a similar study, it was found that there was a positive relationship between the orexin and triglyceride levels in the perifornical hypothalamus (13).

It has been stated that fatty acids have an increasing potential in orexin synthesis. It has also been determined that there is a positive relation between orexin neurons and lipid circulation (13,17).

Taking into consideration the role of orexin in sleep and the effects of dietary fatty acid intake on its synthesis, this trial was planned by considering that the supplementation of essential fatty acids would increase the orexin levels in patients with narcolepsy and would be able to help the clinical complications of patients with narcolepsy.

Materials and methods

This trial began with 19 males with narcolepsy and 16 healthy males. In accordance with the protocol, 17 narcoleptic patients and 14 healthy males completed the trial. According to the study protocol, those with an allergy to or side effects from omega-3 (n-3) fatty acids and those using hormone therapy would be excluded. Since regular medicine usage, including supplementary vitamin and mineral pills, and hormone replacement therapy may alter hormone levels, patients on a regular medicine regime were also excluded from the study. Healthy subjects were examined via polysomnographic sleep tests to assess

whether they had undiagnosed narcolepsy. For these tests, they had to stay at the hospital for 2 nights and for 4 daytime sessions of 20 min each. For this reason, some healthy subjects refused to participate in the study, and this is why the number of control subjects was less than the number of case subjects.

An n-3 fatty acid supplement of 1.4 g was administered daily to the patients and the healthy subjects for 2 months. Anthropometric measurements of the individuals were taken before and after the study. Plasma orexin, fatty acids, and some other biochemical measurements were also analyzed.

For this study, the ethics committee report was received from Gülhane Military Medical Academy (10 June 2008, Number 1491–550–08/1539).

Body weights (kg) and heights (cm) of the individuals were measured and body mass index (BMI; kg/m^2) was estimated. The weight measurements were taken in the fasting state in the mornings while subjects were wearing light clothes. Height was measured using an inflexible steel meter, 2 m in length, while subjects stood with heels, back, and shoulders against a wall, with feet together and head on the Frankfort plane. Waist and hip circumferences were measured 3 times by means of the inflexible meter for reliability of results, and the waist-to-hip ratio was calculated from these values. Basal metabolic rate (BMR), fat mass (kg, %) and free fat mass (FFM; kg) were detected with a Tanita TBF-300M body composition analyzer (Tanita, Illinois, USA).

Blood was taken from the individuals after 12 h of starvation. Plasma glucose, cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), insulin, orexin A, serotonin, and some fatty acids levels were analyzed.

The levels of glucose, cholesterol, triglyceride, HDL-C, LDL-C, and VLDL-C in plasma were measured photometrically with an Olympus AU 2700 biochemistry autoanalyzer using the original Olympus kits (Olympus, Tokyo, Japan), and the levels of plasma insulin were measured with a Roche E-170 device using the original Roche kits (Roche, Mannheim, Germany) (18,19).

The levels of plasma fatty acids (C14:0, C15:0, C14:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:4, C20:5, C22:0, C22:1, C22:6, C24:0, and C24:1) were analyzed using a gas chromatography-mass spectrometer (GC-MS) (20).

The level of plasma serotonin was measured by analysis with ClinRep® HPLC serotonin kits for the serums obtained from the centrifuged blood taken (21). Plasma orexin A levels were analyzed using orexin peptide radioimmunoassay (RIA) kits from Phoenix Pharmaceuticals (California, USA) with a RIA device; the levels were studied 2 times (22).

The mean, standard deviation, median, and minimum and maximum values of data obtained from the individuals in pre- and poststudy measurements were estimated. The intragroup difference between the pre- and poststudy anthropometric and biochemical parameter values of the individuals and the intergroup differences were evaluated according to the Wilcoxon 2-sample test and the Mann-Whitney U test, respectively. The correlation between the values of pre- and poststudy measurements was examined with Spearman's correlation coefficient. For all analyses, $P < 0.05$ was considered statistically significant.

Results

The study was begun with 19 narcoleptic (32.2 ± 7.50 years old) and 16 healthy males (29.7 ± 10.22 years old), but completed with 17 narcoleptic and 14 healthy males. Table 1 shows the arithmetic averages of the ages and anthropometric measurements of the individuals ($\overline{x}$), standard deviations (SD), and median and minimum and maximum values.

As seen in Table 1, at the end of the study, while there was no statistical significance in anthropometric measurements or other body composition values of the patient group ($P > 0.05$), there was a statistically significant increase in the body weight, BMI, waist-to-hip ratio, and body fat mass (kg, %) values of the control group ($P < 0.05$). When the anthropometric measurements and body compositions of the participants were examined, it was determined that there was a difference in heights between the groups, although there was no intragroup difference

Table 1. The anthropometric measurements and body composition values of participants, pre- and poststudy.

		Narcoleptic					Control					
		$\overline{X} \pm SD$	Median	Min-Max	Z	P	$\overline{X} \pm SD$	Median	Min-Max	Z	P	P'
Age (years)		32.2 ± 7.50	30	23-55	-	-	29.7 ± 10.22	25	21-51	-	-	-
Height (cm)		171.8 ± 6.13	172.0	159-183	-	-	176.6 ± 6.13	176.0	164-189	-	-	0.01*
Weight (kg)	1	77.1 ± 15.04	73.4	52.9-107	−8.77	0.38	75.5 ± 10.98	75.0	60-92.1	−2.54	0.01*	0.76
	2	75.4 ± 13.74	71.7	54.9-99.3			75.1 ± 11.17	73.4	60.5-92.4			0.95
BMI (kg/m²)	1	26.0 ± 4.41	26.1	17.9-37.20	1.01	0.31	24.1 ± 3.20	25.0	19.3-29.7	−2.56	0.01*	0.19
	2	25.4 ± 3.55	25.5	18.6-32.1			24.2 ± 3.39	25.2	19.3-29.8			0.35
WC (cm)	1	90.8 ± 11.20	92.0	69.8-108.5	−1.21	0.22	85.4 ± 10.15	87.0	74-107	−1.05	0.29	0.11
	2	90.1 ± 10.61	88.0	74.1-109			85.4 ± 11.32	84.6	74-109.5			0.20
HC (cm)	1	103.6 ± 8.86	101.0	92-121	−0.71	0.47	101.7 ± 5.99	100.7	94-110.5	−0.77	0.44	0.56
	2	103.1 ± 7.67	101.0	92.5-117			100.8 ± 6.60	101.0	92-109.5			0.41
Waist-to-hip ratio	1	0.87 ± 0.07	0.88	0.75-0.98	−0.59	0.55	0.83 ± 0.06	0.82	0.75-0.97	−2.10	0.03*	0.09
	2	0.87 ± 0.06	0.86	0.77-0.99			0.84 ± 0.06	0.82	0.73-1.0			0.20
Fat mass (%)	1	20.9 ± 6.32	20.4	9-33	−0.42	0.67	17.1 ± 5.94	18.0	8.4-28.5	−3.29	0.001*	0.12
	2	20.3 ± 5.73	19.2	10.5-31.6			17.7 ± 6.51	18.1	9.6-29.1			0.22
Fat mass (kg)	1	16.8 ± 7.64	17.6	4.8-35.5	−0.14	0.88	13.4 ± 6.21	14.9	5.4-26.3	−3.30	0.001*	0.28
	2	15.7 ± 6.25	14.5	5.8-29			13.9 ± 6.79	14.8	5.9-26.9			0.40
Free fat mass (kg)	1	60.3 ± 8.52	59.4	40.1-75.6	−0.80	0.42	62.0 ± 6.02	62.7	51.7-74	−0.07	0.93	0.47
	2	59.7 ± 8.92	58.8	39.5-77.4			61.1 ± 5.78	60.6	52.9-74.3			0.62
BMR (kcal)	1	1768 ± 245.04	1713	1356-2232	−0.80	0.42	1790 ± 167.55	1814	1512-2099	−2.45	0.01*	0.55
	2	1744 ± 229.84	1683	1368-2138			1776 ± 163.81	1799	1531-2127			0.52

* = $P < 0.05$; P = intragroup P-value; P'= intergroup P-value

($P < 0.05$). At the end of study, while there was no statistically significant difference in BMR levels in the patient group ($P > 0.05$), there was a statistically significant increase in BMR levels of the control group ($P < 0.05$).

In pre- and poststudy measurements, some biochemical parameter values of the participants were examined and are shown in Table 2. It was determined that there was an increase in the plasma insulin levels and a decrease in the plasma serotonin levels of the patient group ($P < 0.05$). While the statistical significance of the levels of plasma triglyceride, HDL-C, and VLDL-C between the groups at the onset of the study was identified, a significant difference in the levels of only plasma triglyceride, VLDL-C, and serotonin was observed at the end of the study ($P < 0.05$).

It was detected that the pre- and poststudy median values of plasma orexin levels of the patient group were lower than those of the control group. However, the difference was not significant ($P > 0.05$). Table 3 shows the plasma fatty acid levels of the participants pre- and poststudy. As seen in Table 3, a significant difference in the plasma C17:1, C20:1, C22:1, C24:1, and C22:6 levels of the patient group was detected ($P < 0.05$) at the end of the study. This statistical difference was not observed in the control group ($P > 0.05$). There was a statistically significant intergroup difference in the plasma C16:1, C17:1, C20:4, and C22:6 levels at the onset of the trial and in the plasma C22:1, C24:1, and C22:6 levels at the end of the trial ($P < 0.05$).

As seen in Table 4, the correlation between plasma orexin levels and n-3 fatty acids for C20:5 and C22:6 in the control group was detected at the onset of the trial ($P < 0.05$). A correlation between plasma orexin level in the patient group and plasma C16:1, C20:1, and C22:0 levels was observed at the end of the study ($P < 0.05$).

Discussion

Narcolepsy is a chronic sleep disorder. The disease prevalence in the European Union and the USA are 0.02% and 0.07%, respectively (3,5). There is no information about the prevalence of the disease in Turkey. Because of this, patients ignore the disease, do not know where the essential centers for diagnosis are, and do not have the opportunity to visit these centers. This study was begun with 19 narcoleptic (32.2 ± 7.50 years old) and 16 healthy males (29.7 ± 10.22 years old), but fully completed with only 17 narcoleptic and 14 healthy males (Table 1). Limitations of the study may include a relatively small sample size. Our strict inclusion/exclusion criteria for recruiting homogeneous subjects may be in part responsible for this.

The relationship between ideal body weight and rapid eye movement (REM) and nonrapid eye movement (NREM) in narcoleptics was demonstrated in this study. Depending on the disorders in REM-NREM sleep, it has been reported that patients may become obese (23).

In the clinical studies performed, it was detected that a high BMI level in patients with narcolepsy was more common than in the healthy subjects. A study by Chabas et al. (24) determined that the BMI values of 13 narcoleptics were higher (25.8 kg/m^2) than those of 9 healthy individuals (22.9 kg/m^2). In this study, a statistically significant difference between the groups was not found. The BMI values of the patients with narcolepsy (prestudy: 26 kg/m^2, poststudy: 25.4 kg/m^2) were within normal ranges, as were the BMI values of the participants in the control group (prestudy: 24.1 kg/m^2, poststudy: 24.2 kg/m^2) ($P > 0.05$).

As mentioned above, the tendency toward obesity as well as the high BMI values in patients with narcolepsy could be explained by a low BMR level. In a study by Chabas et al. (24), it was observed that BMR was lower in 13 narcoleptic patients than in 9 healthy individuals ($P > 0.05$). It was determined that BMR values of the patients in this study were within the normal ranges and a difference in BMR levels was not observed in the patients at the end of the study ($P > 0.05$).

Williams et al. (25) administered daily n-3 fatty acid supplements of 2.7 g to 14 healthy individuals for 6 weeks without applying a special diet and determined that there was no difference in the body weights ($P > 0.05$). However, as seen in Table 1, there was an increase in the levels of body fat mass and body weights of the control group after n-3 fatty acid supplementation in this study ($P < 0.05$). In spite of

Table 2. The plasma biochemical measurements of participants, pre- and poststudy.

		Narcoleptic					Control					
		$\overline{X}$ ± SD	Median	Min-Max	Z	P	$\overline{X}$ ± SD	Median	Min-Max	Z	P	P'
Glucose (mg/dL)	1	85.7 ± 9.66	85.0	66-109	−1.63	0.10	89.0 ± 12.75	90.5	64-110	−1.85	0.06	0.28
	2	88.0 ± 10.71	90.0	64-111			82.0 ± 9.47	81.0	69-103			0.06
Total cholesterol (mg/dL)	1	187.4 ± 44.67	180.0	105-276	−0.41	0.67	168.0 ± 29.98	169.0	112-218	−0.72	0.47	0.24
	2	185.0 ± 52.93	170.0	105-271			164.0 ± 25.31	163.0	130-207			0.38
Triglyceride (mg/dL)	1	149.0 ± 74.45	138.0	46-346	−0.87	0.38	100.0 ± 65.26	79.5	40-299	−0.66	0.50	0.01*
	2	151.0 ± 82.30	132.0	57-336			90.0 ± 32.35	79.5	40-146			0.01*
HDL-C (mg/dL)	1	45.3 ± 10.19	45.0	28-78	−0.02	0.97	52.5 ± 6.05	54.0	43-64	−1.85	0.06	0.003*
	2	44.5 ± 8.71	44.0	31-61			49.0 ± 6.63	51.5	37-59			0.11
VLDL-C (mg/dL)	1	33.2 ± 20.81	28.0	14-91	−0.71	0.47	21.0 ± 12.40	17.0	12-60	−0.17	0.86	0.01*
	2	31.4 ± 19.65	26.0	11-87			17.9 ± 6.40	16.0	8-29			0.02*
LDL-C (mg/dL)	1	110.0 ± 36.71	115.0	43-171	−0.61	0.53	95.3 ± 25.02	96.5	53-146	−0.24	0.80	0.22
	2	110.0 ± 36.03	106.0	42-173			97.0 ± 22.89	93.0	67-135			0.26
Orexin (pg/mL)	1	31.5 ± 4.78	30.1	23.2-40.3	−0.26	0.79	31.5 ± 8.56	31.1	14.4-51.3	−0.53	0.59	0.81
	2	30.4 ± 6.05	30.1	14-39.7			30.8 ± 6.36	31.9	14.3-40			0.55
Serotonin (nmol/L)	1	14.5 ± 12.2	9.6	4-43	−2.58	0.01*	18.4 ± 11.86	14.0	6.8-46.6	−1.19	0.23	0.10
	2	10.6 ± 9.22	6.5	2.4-32.7			16.4 ± 11.34	12.0	5.1-36.1			0.04*
Insulin (μU/mL)	1	6.8 ± 3.70	6.8	1.9-12.7	−2.53	0.01*	8.2 ± 5.02	6.9	2.7-21.8	−0.47	0.63	0.52
	2	10.9 ± 7.20	8.8	3.4-32			9.0 ± 4.03	8.2	3.8-17.9			0.66
Plasma Ratio of n-6 to n-3	1	6.7 ± 1.17	7.1	4.7-7.6	−1.604	0.10	4.0 ± 2.96	3.6	0.9-7.8	−0.535	0.59	0.22
	2	2.6 ± 0.92	2.5	1.1-4.2			1.9 ± 0.68	1.9	0.8-3.4			0.04*

* = $P < 0.05$; P = intragroup P-value; P' = intergroup P-value

Table 3. The plasma fatty acids levels of participants, pre- and poststudy.

	Narcoleptic		Control	
	$\overline{X}$ ± SD (Before)	$\overline{X}$ ± SD (After)	$\overline{X}$ ± SD (Before)	$\overline{X}$ ± SD (After)
C14:0	16.83 ± 14.90	17.12 ± 9.53	12.69 ± 6.03	12.40 ± 5.23
C15:0	4.68 ± 2.02	5.08 ± 2.96	4.47 ± 2.07	4.04 ± 0.90
C16:0	408.77 ± 267.08	403.74 ± 212.83	397.70 ± 174.69	362.38 ± 51.02
C17:0	3.67 ± 1.83	3.81 ± 2.09	3.02 ± 1.31	3.37 ± 0.67
C18:0	178.52 ± 128.79	181.83 ± 115.51	144.62 ± 56.35	153.17 ± 28.99
C20:0	0.85 ± 0.35	0.58 ± 0.29	0.65 ± 0.28	0.60 ± 0.23
C22:0	1.36 ± 0.60	0.78 ± 0.41	0.64 ± 0.39	0.72 ± 0.34
C24:0	0.70 ± 0.45	0.46 ± 0.35	0.38 ± 0.11	0.30 ± 0.01
C16.1	12.62 ± 6.95 [b]	15.01 ± 7.25	18.59 ± 7.12	18.29 ± 3.91
C17:1	5.05 ± 1.15 [a, b]	6.72 ± 2.88	5.49 ± 1.38	5.23 ± 0.53
C18:1	505.11 ± 261.51	487.41 ± 248.47	516.9 ± 237.14	443.82 ± 58.40
C20:1	4.05 ± 1.06 [a]	5.54 ± 2.32	4.95 ± 1.93	4.25 ± 0.73
C22:1	5.92 ± 1.65 [a]	8.50 ± 3.78 [b]	6.72 ± 1.80	0.42 ± 0.76
C24:1	2.82 ± 0.81 [a]	4.51 ± 1.98 [b]	3.56 ± 1.05	3.49 ± 0.74
C18:2 (n-6)	36.96 ± 85.48	111.48 ± 52.09	116.30 ± 44.81	103.79 ± 12.22
C20:4 (n-6)	13.45 ± 11.46 [b]	17.38 ± 17.29	23.45 ± 14.05	17.69 ± 5.24
C18:3 (n-3)	1.18 ± 0.83	1.26 ± 0.73	1.4 ± 0.31	1.35 ± 0.40
C20:5 (n-3)	8.57 ± 6.28	11.70 ± 6.29	12.11 ± 8.43	10.44 ± 3.78
C22:6 (n-3)	11.38 ± 7.59 [a, b]	55.79 ± 42.00 [b]	50.54 ± 45.82	63.67 ± 25.92

a: P < 0.05 for intragroup values; b: P < 0.05 for intergroup values

Table 4. The correlation of plasma orexin levels with some plasma fatty acids of participants, pre- and poststudy.

	Narcoleptic				Control			
	Before		After		Before		After	
	r	P	r	P	r	p	r	P
N-3 fatty acids (total)	0.800	0.10	–0.126	0.69	–1.000	0.03*	–0.209	0.53
C16:1	0.120	0.64	–0.674	0.006*	–0.121	0.68	–0.323	0.26
C20:5	0.115	0.65	–0.093	0.74	–0.55	0.04 *	–0.262	0.36
C20:1	–0.012	0.96	–0.530	0.04*	–0.257	0.37	–0.080	0.78
C22:6	0.424	0.10	–0.379	0.16	–0.547	0.04*	0.055	0.85
C22:0	0.407	0.16	–0.829	0.02*	–0.685	0.09	–0.200	0.80

*P < 0.05, Spearman

this, there was no statistically significant difference in the BMI values or body weights in the patient group at the end of the study (P > 0.05) (Table 1).

It is known that the risk of abdominal obesity is high in patients with narcolepsy (3). In the studies performed, as only BMI findings were evaluated and data such as waist and hip circumstances or waist-to-hip ratio were not assessed, there were various differences in interpretations (26). The waist circumference (WC) measurement can give some information about regional fat distribution. The ideal WC measurements are 94 cm or 102 cm in males, but values above 102 cm demonstrate increased cardiovascular risk and fat accumulation in the abdominal area (27). In this study, there was no significance found in the WC measurements. A change in this value was not seen at the end of study (P > 0.05, Table 1). Contrary to these results, Kok et al. (26) determined that the WC values were 101 cm in males and 91 cm in females among 138 patients with narcolepsy. It was also found that patients with those WC values at high risk and at normal risk were 39% and 36%, respectively.

At the same time, the waist-to-hip ratio is the simplest indicator of fat distribution, and this ratio provides information about the amount of abdominal fat. There is a relationship between this ratio and chronic diseases (27). In this study, it was seen that waist-to-hip ratio was within the normal limits in both groups, both before and after the study (Table 1). In a study by Chabas et al. (24), it was detected that there was no difference in the waist-to-hip ratio of the patient and control groups (P > 0.05). Similarly, it is seen in Table 1 that there was no difference in the waist-to-hip ratio between the groups in this study (P > 0.05).

After the studies mentioned above showing that the plasma and CSF orexin levels in narcoleptics are lower than those in healthy individuals, the level of orexin was accepted as a diagnostic criterion for the disease. The mean plasma orexin A levels of the participants pre- and poststudy were found to be 31.5 pg/mL and 30.4 pg/mL in the patients and 31.5 pg/mL and 30.8 pg/mL in the control group, respectively. It was determined that the pre- and poststudy median values of plasma orexin A levels of the patient group were lower than the values for the control group, but the difference was not significant (P > 0.05, Table 2). In a study similar to this study, by Dalal et al. (28), it was determined that plasma orexin A levels in 11 narcoleptic and 20 healthy individuals without narcolepsy were similar within the groups (P > 0.05). A study by Nishino and Mignot (29) identified that the plasma orexin A level was 11-25 pg/mL in 12 patients with narcolepsy and 20-33 pg/mL in 12 healthy individuals; they did not find a statistical significance at this level between the 2 groups (P < 0.05).

In healthy individuals whose ages and BMI values were similar to the subjects of this study, Igarashi et al. (30) observed that plasma orexin A levels were close to those found in our study.

As seen above, while the analysis results in some of the studies are similar to those of this study, some of them are different. This may because plasma orexin A levels are influenced by the genetic factors of the individuals, their sex, their age, and body composition. At the same time, the number of studied individuals and the period of the disease can also cause different plasma orexin A levels.

In this study, plasma glucose, cholesterol, triglyceride, HDL-C, VLDL-C, LDL-C, and insulin levels, as well as plasma orexin A, were analyzed. In various studies, it was reported that the lipid profile of the individuals changed with n-3 fatty acid supplementation. Williams et al. (25) administered n-3 fatty acid supplementation of 2.7 g daily to 14 healthy individuals for 6 weeks without applying a special diet. As a result of the study, plasma triglyceride concentration decreased (P < 0.05) and changes in the levels of LDL-C, VLDL-C, and HDL-C were not observed (P > 0.05).

In another similar study, an n-3 supplement was administered twice a day to 14 patients for 2 months (378 mg eicosapentaenoic acid/capsule [EPA], 249 mg docosahexaenoic acid/capsule [DHA]), and results were compared with those of 13 healthy individuals receiving a placebo (31). Similar to this study (Table 2), it was observed that there was no change in plasma lipid profiles (cholesterol, triglyceride, HDL-C, VLDL-C, LDL-C) within the group at the onset of the study compared to the end of the study (P > 0.05). While the statistical significance in the levels of plasma triglyceride, HDL-C, and VLDL-C at the onset of the study was identified among the groups,

the difference in the levels of only plasma triglyceride and VLDL-C at the end of the study was considered significant ($P < 0.05$).

When Williams et al. (25) administered an n-3 fatty acid supplementation of 2.7 g daily to 14 healthy individuals, they detected that there was no change in the levels of plasma glucose and insulin after 6 weeks ($P > 0.05$). In another similar study, it was observed that there was no difference in plasma glucose levels between 7 individuals receiving 1.5 g of DHA supplementation daily for 9 weeks and the control group at the end of study (32). In this study, a change in the levels of plasma glucose was not observed between the groups from the onset to the end of the study ($P > 0.05$). In the patient group, the increase is considered to result from the enhancing effect of the systemic insulin level of n-3 supplementation received during the study.

It was determined that the levels of monoaminergic neurotransmitters, which are dopamine, serotonin, and norepinephrine, decreased in the frontal cortex region of the brains of laboratory animals fed on insufficient essential fatty acids. When arachidonic acid (AA) and DHA were added to the diet, the decrease in these monoaminergic neurotransmitters was prevented (16). In this study, although plasma orexin A levels of the patients did not change after the administration of n-3 supplementation ($P > 0.05$), it was seen that there was a decrease in serotonin levels in the patients with narcolepsy at the end of the study, in contrast to other studies, and there was a difference in plasma serotonin levels between the patient and control groups as a result of the study ($P < 0.05$, Table 2). In this study, the level of serotonin in the brain was not researched; it is considered that the serotonin level in plasma decreases with the increase of serotonin in some parts of the brain. A large amount of polyunsaturated fatty acid intake increases the viscosity of the membrane, and this also affects the protein structures in the membrane and their functions. It has been announced that a large amount of polyunsaturated fatty acid intake, especially in the brain, causes the reduction of the proteins carrying serotonin (33). However, if it is remembered that the n-3 fatty acids administered in the study were not in high doses, it is unknown how these fatty acids show the reduction effect on the serotonin carrier proteins and decrease the serotonin concentration.

Maximum therapeutic effects of EPA and DHA received in high pharmacological doses have been examined in many clinical trials. Due to the mistakes in food consumption records for the determining amounts of dietary EPA and DHA intake, some problems arose in these studies. It was stated that high doses of EPA and DHA are inappropriate for administration into the blood directly because they could cause some metabolic abnormalities; dietary supplementation was reported to be more reliable (34). However, fish oil supplementation including a pharmacological dose of n-3 is suggested to be used in the long term. It generally includes 1 capsule of 300 or 500 mg of n-3 fatty acid, and it is stated that approximately 4-6 g of n-3 fatty acid intake (15-20 capsules/day) will cause clinical adverse effects. The study results showed that the usage of fish oil supplementation, including EPA and DHA, did not cause major adverse effects, but there were gastrointestinal system symptoms in some individuals because of the number of capsules and their sizes (35). During this trial, an adverse effect of supplementation of fish oil including n-3 fatty acids (760 mg of EPA, 320 mg of DHA) was not observed. Similar to the size of the dose in the study, an adverse effect of the supplement was not observed in other various clinical trials in which n-3 was administered. Peet and Horrobin (36) observed an adverse effect frequency of 16% in the patients to whom they administered 1, 2, and 4 g of EPA.

After the dietary intake of EPA and DHA, their levels are enhanced in plasma and the brain. When EPA is received in lower doses than DHA, its level in plasma increases, whereas the level of DHA in the plasma increases with approximately 2 g of daily intake (34). In a metaanalysis of 12 different studies in which plasma DHA level was examined after DHA supplementation, it was determined that plasma DHA level increased depending on the dose after the administration of 0.2-6 g of DHA during 1-6 months (34). However, in some of the clinical studies examined, it was indicated that the plasma DHA level does not completely reflect the brain or synaptic DHA density. After DHA supplementation, it was observed that plasma DHA levels and plasma EPA levels increased. A fatty acid increase of about 0.4 g/100

g in EPA concentration is observed after a daily 1-g dietary DHA intake. In the same metaanalysis study, an increase in plasma EPA and DHA concentrations after daily 4-g EPA supplementation was detected. In the studies performed, it was determined that plasma EPA and DHA levels increased after EPA and DHA intake, as well as after fish oil supplementation including these fatty acids (34).

In a study by Hamazaki (37), 41 students took either DHA-rich oil capsules containing 1.5-1.8 g of DHA each day (17 females, 5 males) or control oil capsules containing 97% soybean oil plus 3% fish oil (12 females, 7 males) for 3 months. At the end of the study, compared with the onset of the study, there were statistically significant differences in the plasma EPA level of the control group and in the plasma C16:0, C18:1, EPA, and DHA levels of the other group ($P < 0.05$). Similarly, as seen in Table 3, at the end of this study compared with the onset of the study, DHA levels in the plasma of the patient group increased and significant differences in plasma C17:1, C20:1, C22:1, and C24:1 levels were detected ($P < 0.05$). In the control group, a significant change was not observed for plasma fatty acids at the end of the study compared with the onset of the study ($P > 0.05$). Statistically significant differences in the levels of C22:1, C24:1, and C22:6 at the end of the study and of C16:1, C17:1, C20:4, and C22:6 in the plasma at the onset of the study between the groups were determined in this study ($P < 0.05$).

It has been stated that orexin neurons such as some other hypothalamic neuropeptides are positively related to lipid circulation. In the paraventricular nucleus and perifornical hypothalamus, orexin production is stimulated by the dietary fat intake and fat circulation (13,17). Dziedzic et al. (38) identified that prepro-orexin mRNA expression in the lateral hypothalamus increased in rats fed with large amounts of n-3 compared to those fed small amounts of n-3 fatty acids.

In a study by Chang et al. (13), it was detected that orexin expression increased by 20% due to the injection of a 20% fat solution in rats with normal weight. In a similar study, investigators detected that orexin mRNA expression in the lateral hypothalamus increased in rats fed a high-fat diet (73.7% of the energy) (12).

In this study, a correlation between plasma orexin A levels and plasma n-3 fatty acids was observed at the onset of the study in the control group ($r = –1.00$). It was interesting to note that there was a very low correlation level at the end ($r = –0.209$). Similarly, with C22:6, at the beginning there was a negative and significant correlation ($r = –0.547$), whereas at the end, it was positive and insignificant ($r = 0.055$). In the narcoleptic group, orexin showed a positive, high correlation with n-3 fatty acids ($r = 0.80$), whereas at the end, it was negative and very low ($r = –0.126$) (Table 4). These results could be related to the amount of dietary fatty acid intake in the patient and control groups. The dietary n-3 fatty acid intake can alter the results and the correlations.

It can be seen in Table 4 that a correlation between plasma orexin A levels and some fatty acids was observed after n-3 supplementation. We possess complete information related to the effects of fat and fatty acids on hypothalamic peptide systems such as orexin (13). At the same time, as orexin has only been recently discovered and is known as a hypothalamic peptide, it is difficult to compare our study findings with other study results. In other studies performed, the plasma orexin A level was not taken into account. This made it difficult to interpret the strength of our study and to compare the results with those of other studies.

Conclusion

It was observed that a daily dose of 1.4 g of n-3 fatty acid supplement did not increase plasma levels of orexin A over the course of 2 months, but it did affect some other biochemical parameters. The results of the current study should be considered as preliminary findings. Future studies with a larger sample size, longer duration of study, and more doses of supplements are necessary for obtaining clear results.

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